REMARKS/ARGUMENTS

Application Status

Claims 1-6 and 8-20 are pending in the subject application. Claim 1 has been amended to include culture times of the stem cells prior to adding the test agents and the time for harvesting the cells to determine any tissue specific differentiation. Claims 3, and 5-6 have been amended to reflect dependency on claim 1. Claims 2, 4, 7 and 9-13 have been cancelled without prejudice. No new matter has been added by virtue of these amendments and their entry is respectfully requested.

Claim Rejections Under 35 U.S.C. §112

Claims 1-6, 8, and 10-20 are newly rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 has been amended to remove reference to "naïve embryonic stem cell." The term "naïve" is implicit in the specification, that is, an embryonic cell that is isolated from a murine embryo and is not manipulated in any way until the cells are cultured in the differentiating medium as shown in Example 1 and figure 1. Figure 1 is an illustration of the general method wherein an embryonic stem cell is cultured, as a first step for three days in differentiating medium. However, in order to expedite prosecution, Applicants have amended the claims to recite "embryonic stem cells (ES)." Support for this amendment is found throughout the specification. See, in particular Example 1, page 12 and Figure 1.

Claims 2-6, 8, 10 were rejected for reciting the term "stem cell" or "stem cells." Applicants have cancelled claims 2 and 4. Claims 3, 5 and 6 have been amended to depend on claim 1 and the claims recite "stem cells."

Claims 10-12 were rejected as reciting time limits outside the range claimed in claim 1. Applicants have cancelled claims 9-12. Applicants have amended claim 1, to recite the time frames as shown in Example 1, Figures 1 and 2 and Example 2, page 16, lines 9-16.

Claims 2-6, 8 and 20 were rejected for depending on rejected base claims and for lack of clarity. Applicants have cancelled claims 2 and 4. Claim 6 was amended to depend on claim 1. Claim 1 was amended to explicitly state that which is implicit.

No new matter has been added by virtue of these amendments and their entry is respectfully requested. In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 112

Claims 1-6, 8, and 10-20 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Applicants respectfully traverse. However, in order to compact and expedite prosecution Applicants have amended claim 1.

Claim 1 has been amended to remove reference to "naïve embryonic stem cell." The term "naïve" is implicit in the specification, that is, an embryonic cell that is isolated from a murine embryo and is not manipulated in any way until the cells are cultured in the differentiating medium as shown in Example 1 and figure 1. Figure 1 is an illustration of the general method wherein an embryonic stem cell is cultured, as a first step for three days in differentiating medium. However, in order to expedite prosecution, Applicants have amended the claims to recite "embryonic stem cells (ES)." Support for this amendment is found throughout the specification. See, in particular Example 1, page 12 and Figure 1. Applicants have also amended claim 1, to recite the time frames as shown in Example 1, Figure 1 and Example 2, page 16, lines 9-16 and have removed the reference to the "3 day culturing" as shown on page 13, lines 18-21. For example, page 16, lines 9-16 teach:

> Based on these previous reports for embryonic liver development, growth factors and cell culture matrix were applied to induce hepatic maturation of EBs *in vitro* (Figure 2). Initially EBs were attached to collagen coated culture plates at Day 5 *in vitro* differentiation. As an early stage factor potentially inducing hepatic differentiation, aFGF was added from **Day 9 to Day 12**. From **Day 12 to Day 18**, HGF was added as a mid-stage factor. Oncostatin M (OSM), dexamethasone (Dex) and a mixture of insulin, transferrin and selenious acid (ITS) were added as late-stage factors from **Day 15 to Day 18.** The patterns of hepatic lineage gene expression were analyzed at Day 18. (Emphasis added).

On page 9, lines 24-31 through to page 10, lines 1-2:

> Other suitable culture conditions for promoting differentiation of stem cells include placing the cultures in a humidified, 5% carbon-dioxide containing incubator, maintaining the temperature at about 37°C (e.g., between 35-39 C) for murine or human stem cells. In general, after being contacted with the substances being screened, the subcultures are cultured under conditions that promote differentiation for **about 7-14** days prior to being analyzed for modulation of gene expression. This time period may vary depending on the particular type of stem cells used and the particular differentiation pathway being analyzed. For example, in assays utilizing murine ES cells, for differentiation into cardiac myocytes, changes in gene expression may be analyzed before 8-10 days in culture. In comparison, for differentiation into hepatocytes, changes in gene expression may be analyzed before 15-18 days in culture. (Emphasis added).

Figure 2 shows the over view of the *in vitro* differentiation assay, wherein differentiating factors are only added on day 5 of culture. Therefore, the phrase, in the currently amended claim 1: "culturing the first and second subcultures for at least about 3 days in the absence of a test substance" prior to the addition of a test substance is clearly taught by Applicants.

Applicants have amended the claims to explicitly describe the invention. Amendment and cancellation of the claims are not to be construed as an acquiescence to any of the rejections/objections set forth in the instant Office Action, and were done solely to expedite prosecution and allowance of the application. Applicants reserve the right to pursue the claims as originally filed, or substantially similar claims, in this or one or more continuation patent applications.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant invention.

Rejections Under U.S.C. §103

Claims 1-4 and 10-12 remain rejected and claims 6, 8, 14-19 under 35 U.S.C. § 103(a) as being unpatentable over Liu, and further in view of U.S. Patent No. 5,874,301 to Keller.

Applicants respectfully traverse.

Applicants invention, is directed in part to agent-induced gene changes of stem cells that are expressed in a tissue-specific manner. Applicants have amended claim 1 to include the number of days in which to culture the cells prior to addition of the agent and the length of time that the cells remain in culture after addition of the agent. Support for the amendment is found throughout the specification and a schematic illustration of the method is shown in figures 1 and 2. The stem cells disclosed by applicants **are not committed to, nor predisposed to,** any specific cell lineage. (See, for example page 3, lines 7-8. Emphasis added). Regarding the steps disclosed by applicants in claim 1, applicants teach the steps of culturing of embryonic stem cells (*i.e.*, totipotent stem cells) followed by sub-culturing of the stem cells prior to the addition of any agent. Thus, applicants do not merely isolate stem cells and culture but culture the cells in media lacking any factor that may induce differentiation.

Lui *et al.*, does not teach or contemplate the method disclosed by Applicants. The Examiner asserts that it would be obvious to one of skill in the art "that time is needed" for the stem cells to differentiate. However, it is not obvious to one of skill in the art that different lineages vary in the time to differentiate. This is not taught by Lui *et al.* In contrast, Applicants teach that a stem cell which will differentiate into a hepatocytes, by addition of certain factors, requires that each factor be added on certain days of the differentiation pathway. See for example, page 15, lines 19-27:

> Undifferentiated ES cells did not express these endodermal or hepatocyte lineage genes- Figure 2 depicts the *in vitro* ES differentiation procedure used in this study. Figure 3 illustrates the pattern of endodermal specific gene expression in differentiating EBs without additional growth factors. Transthyretin was expressed **within 6 days** after removal of the LIF. Alpha-fetoprotein and alpha 1-antitrypsin were expressed **within Day 9.** Albumin mRNA expression first appeared **within Day 12. Late differential markers** of hepatocyte, TAT and G6P were not detectable throughout the time course (up to Day 18). These data indicate that ES cells spontaneously differentiate toward hepatic or yolk sac lineage cells, but they do not differentiate into mature hepatocytes. (Emphasis added).

On page 16, lines 11-29:

> Initially EBs were attached to collagen coated culture plates at Day 5 *in vitro* differentiation. As an **early stage factor** potentially inducing hepatic differentiation, aFGF was added from **Day 9 to Day 12.** From **Day 12 to Day 18,** HGF was added as a **mid-stage factor.** Oncostatin M (OSM), dexamethasone (Dex) and a mixture of insulin, transferrin and selenious acid (ITS) were added as **late-stage factors from Day 15 to Day 18.** The patterns of hepatic lineage gene expression were analyzed at Day18.
>
> As shown in Figure 4, a combination of these growth factors enhanced the expression of albumin mRNA, which is an indicator of hepatocyte maturation. The expression of albumin was increased 9.5-fold and 7.4-fold (real-time PCR) by the growth factors on collagen-uncoated culture and collagen-coated culture, respectively. Moreover, G6P and TAT genes, indicators of hepatocyte maturation, were now expressed in EBs in the presence of the growth factors. It appeared that collagen coating further enhanced the expression of G6P and TAT.
>
> The **effects of growth factors at individual stages** on hepatic development using EBs plated on collagen coated dishes were also examined. As demonstrated in Figure 5a, the **mid- stage factor** (HGF) or **late stage factors** (OSM, Dex, ITS) were critical for G6P expression. The late-stage factors exclusively enhanced TAT gene expression. Although Dex, by itself, slightly induced TAT expression, the mixture of the late stage factors mostly enhanced the TAT expression (Figure 5b). (Emphasis added).

In contrast to applicants' invention, Liu '535 does not teach any time frame for culturing cells prior to adding a test agent, nor the amount of time needed to culture stem cells in the presence of a test agent or which test agents to add at which time frames. It is not obvious from one of ordinary skill in the art to determine from Liu *et al.*, whether stem cells would have to be

isolated and cultured for any period of time prior to addition of an agent and for how long they should be cultured in the presence of an agent, or when the agent should be added, in order to determine tissue specific differentiation. Undue experimentation would be required under Liu *et al* to teach what applicants teach. It is also not obvious from Liu *et al.*, whether totipotent stem cells vs. hematopoietic stem cells would need to be cultured over any specific time periods prior to addition of, during addition of an agent and when the agent should be added. It is also not obvious from the reading of Liu whether any totipotent stem cell could even be manipulated to differentiate from a totipotent stem cell to a pluripotent stem cell to a pre-committed stem cell, such as a hematopoietic stem cell.

Regarding the Examiner's allegations that "in view of Liu, one of ordinary skill in the art at the time of invention by Applicant would have been motivated to identify drug candidates for promoting tissue-specific differentiation of a stem cell by providing a number of test substances (otherwise there would be no pool of substances from which to identify a substance that works), and culturing cells *in vitro* in the presence of each substance, individually, under conditions that allow for such differentiation, and analyzing the cells in cultures for increased tissue-specific gene expression markers." Applicants respectfully traverse.

Applicants' claim 1 is directed in part to "at least a first test substance and a second test substance, the first and second test substances having different molecular structures." These first and second test substances with different molecular structures are then administered to stem cells that are first sub-cultured, as discussed above, at least three days prior to exposing each sub-culture to the first and second test substances of different molecular structures. Furthermore, the Examiner admits that "Liu does not teach the aspects of culturing cells ....." (see page 7, 1st paragraph of the Office Action dated 4/08/2004).

The Examiner alleges, in the paragraph bridging pages 9 and 10, that:

> In view of Liu, one of ordinary skill in the art at the time of invention by Applicant (hereinafter the "Artisan") would have been motivated to identify drug candidates for promoting tissue-specific differentiation of a stem cell as taught by Liu, by providing a number of

> test substances (otherwise there would be no pool of substances from which to identify a substance that works), and culturing cells *in vitro* in the presence of each substance, individually, under conditions that allow for such differentiation, and analyzing the cells in the cultures for increased tissue-specific gene expression markers using the embryonic stem cells of Keller. The Artisan would have been motivated to do so in order to identify agents that cause the differentiation of such embryonic stem cells into, *inter alia*, leukocytes and erythrocytes. Moreover, the Artisan would have had a reasonable expectation of success, as Liu had already shown that such screens could work *in vivo*, and culture techniques for the specific cells had already been demonstrated by Keller.

Applicants respectfully traverse

Keller does not teach, either directly or indirectly the subject matter of the instant application. Keller et al., do not teach the addition of factors to the assay at specific times, as taught by applicants. Furthermore, the so-called "3 day pre-culturing" in column 7, paragraph 1, Keller et al, teach the culturing of cells in the **presence of differentiating factors**. See for example col. 6., lines 39-64:

> In one embodiment, an EB cell population of the present invention is derived by culturing a population of ES cells in an embryoid body medium, which is **medium that stimulates the differentiation of an ES cell population to an EB cell population.** Typically, an ES cell population is maintained in an undifferentiated state by culturing the cells in an embryonic stem cell medium including leukemia inhibitory factor (LIF) and fetal calf serum (FCS). To produce an EB cell population in accordance with the present invention, an ES cell population is removed from the embryonic stem cell medium and re-cultured in embryoid body medium in which the LIF and the FCS have been replaced by either **PP-FBS or normal FCS pre-selected for the ability to promote EB cell development (referred to herein as pre-selected normal FCS).** Both the absence of LIF and the presence of PP-FBS or pre-selected normal FCS in the culture medium stimulates the ES cell population to **differentiate** into an EB cell population of the present invention. An embryoid body medium of the present invention includes a suitable amount of PP-FBS or pre-selected normal FCS that is capable of stimulating the differentiation of an ES cell population to an EB cell population. A preferred embryoid body cell medium of the present invention includes from about 5% to about 30%, more preferably from about 10% to about 20%, and even

more preferably about 15% PP-FBS or pre-selected normal FCS. (Emphasis added).

The 3 day "pre-culturing" that Examiner asserts teaches the instant invention does not teach Applicants invention. Applicants teach the culturing of **undifferentiated** stem cells prior to addition of any differentiating factor. The EB cells are **already** at some differentiation stage as taught by Keller, such as for example, by culturing cells with PP-FBS. (See, Keller, col. 6, lines 56-60). Keller, cannot make up for the deficiencies of Liu *et al.*

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant invention.

Claim 5 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Lui and Keller as applied to claim 1 above, and further in view of Kondoh, *et al.*, May 1999) *J. Biochem. Biophys. Meth.*, 39:137-142.

Applicants respectfully traverse.

Lui and Keller have been discussed above and for the sake of brevity and compact prosecution will not be repeated here. Kondoh et al., describe cell clones derived after *in vitro* selection for **homologous recombination** in **six different genes**. Therefore, an assay, as taught by Applicants, would be severely compromised by using any such cell in Kondoh. As discussed above, neither Lui or Keller alone or in combination teach the instant invention. Kondoh *et al.*, does not make up the deficiencies of Lui and Keller because one of skill in the art would not be motivated to substitute the cells of Kondoh for Applicants cells used in Applicants assay. Furthermore, Kondoh *et al.*, discuss aggregation of cells and classify certain cell clones into different classes. One of ordinary skill in the art would not be motivated based on Kondoh to supplement these cells for use in an assay based on Lui and Keller.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant invention.

Claim 13 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Liu and Keller as applied to claim 1 above, and further in view of U.S. Patent No. to Moore, filed 24 June 1992, patented 12 July 1994.

Applicants respectfully traverse.

Applicants have cancelled claim 13, therefore, this rejection is now moot and withdrawal of the rejection is respectfully requested. Applicants however, maintain that Moore is deficient as it does not teach or disclose how to culture and maintain stem cells. Cells disclosed by Moore are **malignant** and normal cells. (See Example 2). As one of ordinary skill in the art is aware, cells of different types require different media and growth conditions, such as culturing time frames. The Examiner is basing the culture of **all** mammalian cells as "taught by Moore." Moore does not teach how to culture **all** mammalian cells, for example, cells like Schwann cells. Moore also does not teach how to culture stem cells by maintaining them in culture in an undifferentiated state until the addition of a tissue specific differentiation agent is added to the culture. Applicants submit that Moore provides absolutely no guidance as to the culturing and maintenance of **stem** cells and cannot make up for the deficiencies of Liu. Accordingly, Liu, in view of Moore, would not provide a reasonable expectation of success and nor would a person of ordinary skill in the art be motivated to combine these two references as Moore's teachings do not extend to all mammalian cells including stem cells.

Claim 20 is rejected under 35 U.S.C. § 103 (a) as being unpatentable over Liu and Keller, as applied to claim 1 above and further in view of U.S. Patent No. 5,143,854 to Pirrung.

Applicants respectfully traverse.

Applicants have discussed the novelty of applicants invention over Liu '535 and Keller. Briefly, Lui *et al.*, does not teach or contemplate the method disclosed by Applicants. The Examiner asserts that it would be obvious to one of skill in the art "that time is needed" for the

stem cells to differentiate. However, it is not obvious to one of skill in the art that different lineages vary in the time to differentiate. This is not taught by Lui *et al.* In contrast, Applicants teach that a stem cell which will differentiate into a hepatocytes, by addition of certain factors, requires that each factor be added on certain days of the differentiation pathway.

In contrast to applicants' invention, Liu '535 does not teach any time frame for culturing cells prior to adding a test agent, nor the amount of time needed to culture stem cells in the presence of a test agent or which test agents to add at which time frames. It is not obvious from one of ordinary skill in the art to determine from Liu *et al.*, whether stem cells would have to be isolated and cultured for any period of time prior to addition of an agent and for how long they should be cultured in the presence of an agent, or **when** the agent should be added, in order to determine tissue specific differentiation. Undue experimentation would be required under Liu *et al* to teach what applicants teach. It is also not obvious from Liu *et al.*, whether totipotent stem cells vs. hematopoietic stem cells would need to be cultured over any specific time periods prior to addition of, during addition of an agent and when the agent should be added. It is also not obvious from the reading of Liu whether any totipotent stem cell could even be manipulated to differentiate from a totipotent stem cell to a pluripotent stem cell to a pre-committed stem cell. such as a hematopoietic stem cell.

Keller does not teach, either directly or indirectly the subject matter of the instant application. Keller *et al.*, do not teach the addition of factors to the assay at specific times, as taught by applicants. Furthermore, the so-called "3 day pre-culturing" in column 7, paragraph 1, Keller et al, teach the culturing of cells in the **presence of differentiating factors.**

The 3 day "pre-culturing" that Examiner asserts teaches the instant invention does not teach Applicants invention. Applicants teach the culturing of **undifferentiated** stem cells prior to addition of any differentiating factor. The EB cells are **already** at some differentiation stage as taught by Keller, such as for example, by culturing cells with PP-FBS. (See, Keller, col. 6, lines 56-60). Keller, cannot make up for the deficiencies of Liu *et al.*

In view of the fact that neither Lui nor Keller, taken either alone or in combination, do not render the instant invention obvious, one of ordinary skill in the art would not be motivated, nor would it be obvious to combine these references to arrive at the instant invention. Pirrung alone does not teach or disclose the instant invention, and without guidance from Applicants, it would not be obvious to one of ordinary skill in the art to analyze the stages of the stem cell differentiation. Applicants submit that Pirrung does not suggest, teach or make obvious in any way **how** to, **when and how** to culture cells, determine **which** RNA species are present and how one of ordinary skill in the art could adapt an RNA molecule for use on a chip.

It is respectfully submitted that for the foregoing reasons, claim 20 is patentable over the cited reference(s) and satisfy the requirements of 35 U.S.C. § 103 (a). As such, these claims are allowable.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-6 and 8-20 is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

This response is accompanied by a petition for a one month retroactive extension of time and the required fee. Although, Applicants believe that no further extensions of time (beyond the one month petition) are required with submission of this paper, Applicants request that this submission also be considered as a petition for any further extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,
AKERMAN SENTERFITT

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